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Efficacy of Influenza Vaccination in Adult Liver Transplant Recipients

N.M.R. Soesman,[1,2] G.F. Rimmelzwaan,[1] N.J. Nieuwkoop,[1] W.E.P. Beyer,[1] H.W. Tilanus,[2] M.H. Kemmeren,[2] H.J. Metselaar,[2] R.A. de Man,[2] and A.D.M.E. Osterhaus[1]*

[1]Department of Virology and National Influenza Center, Erasmus Medical Center, Rotterdam, the Netherlands
[2]Department of Hepatogastroenterology, Erasmus Medical Center, Rotterdam, the Netherlands

To assess the efficacy of influenza vaccination in immunocompromised adult liver transplant (LTx) recipients, the serum antibody responses of 61 of these patients and 35 liver cirrhosis patients with those of 45 of their healthy spouses were compared, after one and two vaccinations with a commercial trivalent subunit influenza vaccine. In addition, virus-specific proliferative T-cell responses were measured in LTx recipients and their healthy spouses. In all three study groups, significant rises in geometric mean antibody titers were observed for all three antigens after one vaccination. These titers did not continue to increase significantly after the second vaccination in patients with cirrhosis and control subjects but did rise for LTx recipients. The overall antibody response to all three influenza virus strains proved to be significantly lower in the LTx recipients than in the group of healthy subjects after both one and two vaccinations. More than 68% of the LTx recipients developed hemagglutination-inhibiting serum antibody titers $\geq$40 against all three vaccine strains after the first vaccination and more than 80% after the second vaccination. These findings correlated with the T-cell responses determined for the group of LTx recipients and healthy control individuals. Testing of the respective serum samples against influenza virus A/Sydney/5/97, which circulated in the 1997–1998 influenza season and showed a considerable mismatch with the vaccine strain A/Nanchang/933/95, indicated that such a mismatch may have significant consequences for vaccine efficacy, especially for LTx recipients. Collectively the data show that LTx recipients can be vaccinated effectively against influenza despite immunosuppressive therapy. A two-dose vaccination regimen improved vaccination efficacy in LTx recipients. Whether transplant patients generally benefit from a two-dose vaccination regimen should be evaluated further. ***J. Med. Virol. 61:85–93, 2000.***

KEY WORDS: influenza vaccination; liver transplant recipients

INTRODUCTION

Influenza viruses are recurring agents of respiratory illness in the general population, producing significant death rates annually in certain risk groups—usually as the result of serious complications, such as pneumonitis, secondary bacterial infections, and heart failure [Aschan et al., 1989; Ljung et al., 1993; Mauch et al., 1994]. Therefore, annual vaccination against influenza is recommended for risk groups, which include patients with cardiac, pulmonary, and renal disease; patients with diabetes mellitus; the elderly; and immunocompromised patients. Although transplant recipients receiving immunosuppressive therapy also belong to this category, the benefit of influenza vaccination in these patients is still the subject of controversy. The efficacy of standard one-dose influenza vaccination in these patients is not clear, since the results obtained in several studies addressing this issue are not consistent.

Vaccine-induced serum antibody responses were impaired severely in solid organ transplant recipients in some studies [Pabico et al., 1976; Rytel et al., 1977; Stiver et al., 1977; Kumar et al., 1978; Huang et al., 1983; Versluis et al., 1986; Blumberg et al., 1996; Admon et al., 1997], while in other studies, in which patients with comparable immunosuppressive regimens were included, the responses were not significantly different from those in healthy controls [Briggs et al., 1972; Caroll et al., 1974; Grekas et al., 1993]. Most of these studies, however, focused predominantly on heart and kidney transplant recipients. Similar studies in liver transplant (LTx) recipients have been limited [Blumberg et al., 1996] or were carried out with pediatric patients [Mauch et al., 1995; Mack et al., 1996]. A complicating factor in the assessment of the efficacy of

*Correspondence to: A. D.M.E. Osterhaus, Department of Virology, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, the Netherlands. E-mail osterhaus@viro.fgg.eur.nl

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influenza vaccination in immunosuppressed individuals is that it is not clear whether the correlation observed between the hemagglutination-inhibiting (HI) serum antibody titers and protection observed in healthy vaccinees also holds true for immunosuppressed individuals. In the present study, we evaluated the immunogenicity of a commercially available subunit influenza vaccine (Influvac 97) by comparing the development of HI serum antibody titers in adult LTx recipients, patients with liver cirrhosis, and their healthy spouses after one and two vaccinations. Moreover, the induction of virus-specific T-cell immunity upon vaccination was studied in a number of LTx recipients by analyzing the virus-specific proliferative T-cell responses with their peripheral blood mononuclear cells (PBMCs) compared with healthy controls. In addition, we evaluated the level of antibody-mediated immunity against influenza virus A/Sydney/5/97 (H3N2), the prototype virus strain of the 1997–1998 influenza epidemic, which showed a mismatch with the vaccine strain used (influenza virus A/Nanchang/933/95).

MATERIALS AND METHODS

Study Population

A total of 144 subjects entered the study. They consisted of 61 liver transplant recipients (32 men and 29 women) between 20 and 68 years of age (average 51), 36 patients with liver cirrhosis (21 men and 15 women) between 27 and 72 years of age (average 54), and 47 healthy spouses (20 men and 27 women) between 16 and 72 years of age (average age, 53). Of these individuals, 34%, 39%, and 21%, respectively, had received an influenza vaccine a year earlier. Primary disease conditions that had led to liver transplantation included primary biliary cirrhosis (n = 12), primary sclerosing cholangitis (n = 8), various kinds of hepatitis (n = 13), liver cirrhosis due to alcohol abuse (n = 6), cryptogenic liver cirrhosis (n = 6), and fulminant liver failure (n = 12).

Transplantations had been carried out 3–127 (average, 38) months before the vaccination study. The LTx recipients were subjected to one of the following combinations of immunosuppressive therapies:

- Thirty-two patients received cyclosporine (median dosage, 187 mg daily; range, 100–450 mg) combined with prednisone (median dosage 7.5 mg daily; range, 2.5–12.5)
- Eleven patients received a combination of cyclosporine (median dosage, 150 mg daily; range, 100–300 mg), azathioprine (median dosage, 75 mg daily; range, 50–150 mg), and prednisone (median dosage 5 mg daily; range, 2.5–12.5 mg)
- Ten patients received cyclosporine only (median dosage, 250 mg daily; range, 150–400 mg)
- The remaining patients recieved a combination of azathioprine (median dosage, 62.5 mg daily) and cyclosporine (median dosage, 150 mg daily) or azathioprine (median dosage, 62.5 mg daily) and prednisone (median dosage, 12.5 mg daily).

The study was approved by the medical ethical committee of the hospital, and written informed consent was secured from all individuals before inclusion in the study.

Influenza Vaccine

A commercially available subunit vaccine (Influvac 97; Solvay Duphar, the Netherlands) was used in this study. The vaccine contained 15 μg hemagglutinin of the following virus strains: A/Nanchang/933/95 (H3N2), A/Johannesburg/82/96 (H1N1), and B/Harbin/7/94, as recommended by the World Health Organization for the 1997–1998 season [Anonymous, 1997].

Vaccination and Sampling

Each subject received a first intramuscular injection of the vaccine as recommended by the manufacturer. After 28 days each subject received a second vaccination. Just before each vaccination and 4 weeks after the last vaccination, blood samples were collected for the preparation of serum and the isolation of PBMCs. From one patient with cirrhosis and two control subjects no blood was collected on days 28 and 56, and from one cirrhosis patient and one LTx recipient no blood sample was available on day 56. The final numbers of serum samples available after one vaccination were 35, 45, and 61 for cirrhosis patients, control subjects, and LTx recipients respectively; after two vaccinations, there were 34, 45, and 60 samples available, respectively.

Hemagglutination-Inhibition Assay and Handling of the Data

The serum samples were stored at −20°C until testing. Influenza virus strains for titration (three vaccine components and A/Sydney/5/97 [H3N2]) were propagated in 11-day-old embryonated chicken eggs. The HI test was performed in duplicate according to standard methods [Palmer et al., 1975; Masurel et al., 1981] with turkey erythrocytes. Ferret sera raised against the test antigens were used as positive controls. All serum samples of one individual were tested simultaneously. For calculations, a titer of 5 was arbitrarily assigned to sera with a titer of < 10. Titers were transformed to a logarithmic scale, and geometric means were used for further calculations. Serologic end-point variables were the geometric mean of postvaccination HI titers (GMT), the proportion of subjects with postvaccination titers ≥ 40 (which are considered to correlate with protection in healthy individuals [Kilbourne et al., 1973; Briggs et al., 1980]), and the proportion of subjects showing an increase in antibody titer equal to or greater than fourfold. The results were analyzed by conventional statistical methods (ANOVA for GMT and χ^2 test for proportion of subjects showing a positive response) and regression models to control for confounding factors, as described previously [Palache et al., 1993]. A P value < 0.05 was considered to indicate statistical significance.

In Vitro T-Cell Proliferative Responses

PBMCs from 24 LTx recipients and 24 controls were obtained by sedimentation of heparinized blood on Ficoll-Isopaque (Vacutainer CPT; Becton Dickinson, Franklin Lakes, NJ). These individuals were selected to obtain a match for vaccination history between the two groups. Ten (41.7%) of the LTx recipients and nine of the control subjects (37.5%) had been vaccinated in the previous year. PBMCs were cultured in round-bottom, 96-well microtiter plates (Costar, Corning, NY) at a density of 10^5 cell per well in 150 μL RPMI culture medium supplemented with 10% (v/v) pooled human serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and 2 mmol/L L-glutamine, essentially as described previously [Van Binnendijk et al., 1989]. Purified membrane glycoprotein subunit preparations of the virus strains A/Nanchang/933/95 (H3N2), A/Johannesburg/82/96 (H1N1), and B/Harbin/7/94 (generously provided by Solvay Pharmaceuticals BV, the Netherlands) were added at concentrations that have been found to be optimal for the specific stimulation of PBMCs obtained from human donors (100–300 ng per well). PBMCs were incubated for 5 days at 37°C, followed by a pulse of 1 μCi [^{3}H]thymidine for 16 hours. Cells were harvested, and the incorporated [^{3}H]thymidine was measured in a scintillation counter (1205 Betaplate; LKB, Uppsala, Sweden). The results were expressed as stimulation indexes (SI), which represent the ratio of the mean proliferation of triplicate cultures after stimulation with the respective antigens to the mean of medium controls. A SI $\geq$ 3 was considered positive for an antigen-specific T-cell response. For statistical analysis the *t*-test was used.

RESULTS

Hemagglutination-Inhibiting Antibody Response Against the Vaccine Strains

The individual antibody titers against the respective vaccine strains before and after one or two vaccinations are plotted in Fig 1. In all three study populations, a significant antibody response was observed after one vaccination against all three vaccine strains (*P* values < 0.001). The highest postvaccination GMTs were found among patients with liver cirrhosis and controls (see Table I). The antibody responses against all three vaccine strains were significantly lower in LTx recipients than those observed in healthy individuals or patients with liver cirrhosis. After the second vaccination, the GMTs in the group of healthy individuals and the patients with liver cirrhosis did not increase further, whereas the GMTs of the group of LTx recipients slightly but significantly increased. The GMTs of the latter group, however, remained significantly lower than in the control group and the group of cirrhosis patients.

Higher proportions of cirrhosis patients and LTx recipients than control subjects had prevaccination antibody titers $\geq$ 40 against the vaccine strains, which is in line with their different histories of previous vaccination (Fig. 2). After one vaccination a significant difference was found between A/Nanchang/933/95-specific and A/Johannesburg/82/96-specific antibody titers $\geq$40 measured in the control group (97.8% and 100%, respectively) and the LTx group (77.0% and 91.8%, respectively) but not for B/Harbin/7/94-specific antibody titers $\geq$ 40. The percentages of individuals with antibody titers $\geq$ 40 against B/Harbin/7/94 after one vaccination were 75.6% in the control group and 68.2% in the group of LTx recipients. After the second vaccination the difference in A/Nanchang/933/95- and A/Johannesburg/2/96-specific antibodies observed between the controls (97.8% and 100%, respectively) and the LTx recipients (88.3% and 93.3%, respectively) was no longer statistically significant. The percentage of individuals with antibody titers $\geq$ 40 against B/Harbin/7/94 was 81.1% in the control group and 80% in the group of LTx recipients. These differences were not statistically significant.

In the group of LTx recipients, the response rates (greater than or equal to fourfold titer rises) between days 0 and 28 also were significantly lower than in the control subjects and the group of patients with liver cirrhosis (Fig. 3). Of the LTx recipients, 57.4%, 55.7%, and 55.7% showed a greater than or equal to fourfold titer rise against the vaccine strains A/Nanchang/933/95, A/Johannesburg/82/96, and B/Harbin/7/94, respectively. These figures were 80.0%, 86.7%, and 84.4%, respectively, in the control group and 60.0%, 65.7%, and 71.4%, respectively, in the group of cirrhosis patients. After the second vaccination the response rate against all the virus strains compared with prevaccination values still increased considerably in the group of liver transplant recipients (63.3%, 63.3%, and 63.3 %, respectively), which was not the case for the control subjects and the cirrhosis patients.

The influence of prevaccination status on the antibody response to vaccination was addressed by two approaches. First, the proportions of subjects with a postvaccination titer $\geq$ 40 were recalculated for the subset of subjects with a prevaccination titer < 40 (Table III). Again, the LTx patients showed lower proportions (ranging from 60.4% to 70.0%) than control and cirrhosis groups (71.8% to 100%) (significant for antibodies directed to A/Nanchang/933/95 and A/Johannesburg/82/96). Second, postvaccination titers were corrected for the confounding factors of prevaccination titers and status of previous vaccinations by an ANCOVA procedure. Both factors significantly influenced postvaccination titers, but after correction results were similar to those shown in Table I (not shown). Thus, also when the level of preexisting antibodies (induced by natural infection or previous vaccinations) were taken into account, the serum antibody response after influenza vaccination is diminished in LTx recipients.

Hemagglutination-Inhibiting Antibody Response to Influenza Virus A/Sydney/5/97

Serum antibody titers also were measured against the epidemic influenza A (H3N2) virus strain, which circulated during the 1997–1998 influenza season. As

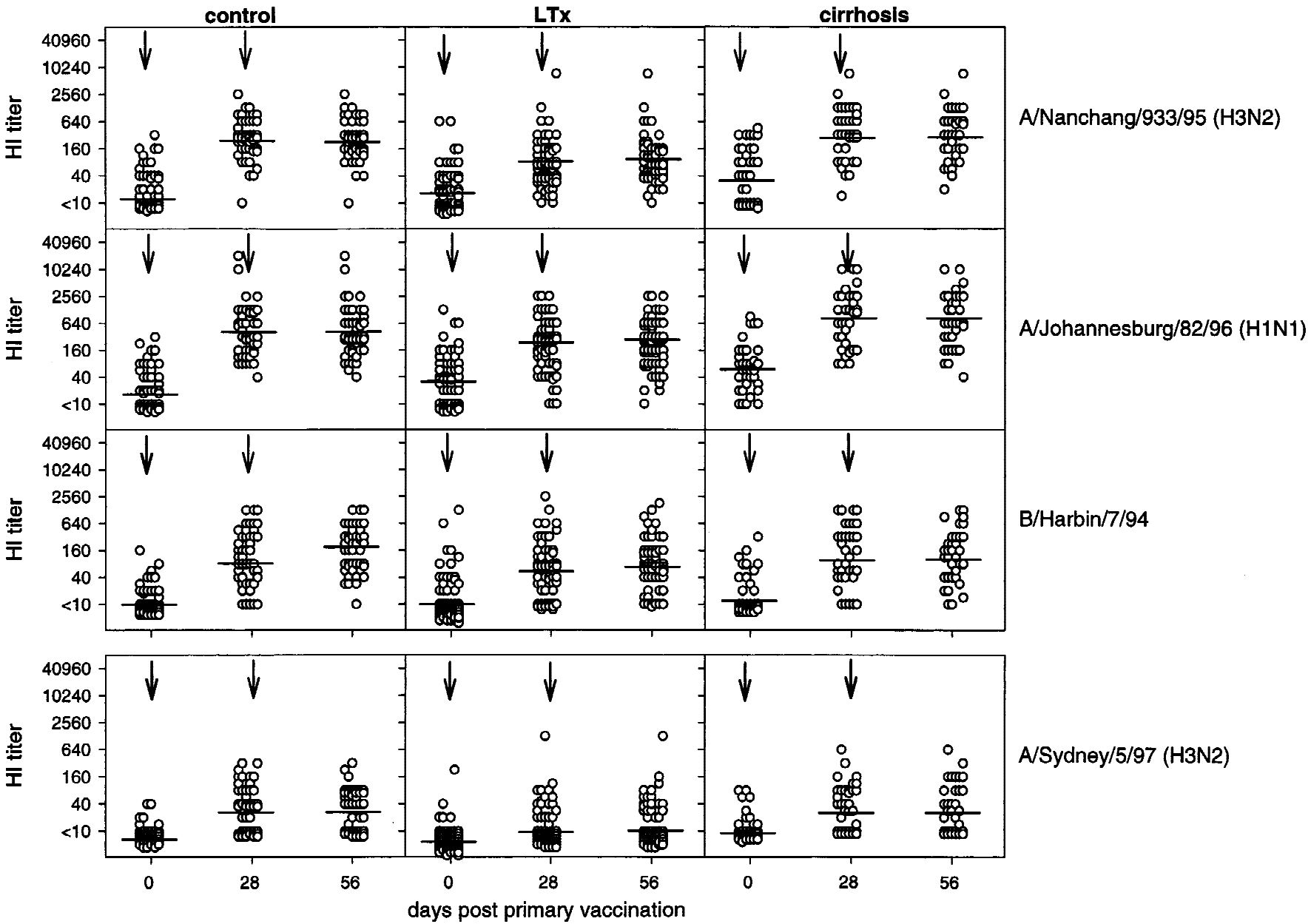

Fig. 1. Individual hemagglutination-inhibiting serum antibody titers in the groups of healthy control individuals, LTx recipients, and cirrhosis patients against the influenza virus vaccine strains A/Nanchang/933/95 (H3N2), A/Johannesburg/82/96 (H1N1), and B/Harbin/7/94 before and after one or two vaccinations and against the epidemic strain A/Sydney/5/97 (H3N2). The arrows indicate the time points of the two vaccinations at day 0 and day 28. The horizontal bars indicate the geometric mean titer of the respective study groups at the indicated time points.

TABLE I. Geometric Means of Hemagglutination-inhibiting Serum Antibody Titers Against Influenza Virus Vaccine Strains

Days after vaccination	Controls[a]	Cirrhosis[a]	LTx[a]	*P* (ANOVA)
A/Nanchang/933/95 (H3N2)				
0	16 (11–24)	34 (19–61)	16 (12–22)	0.025
28	270 (194–375)	331 (207–531)	74 (53–103)	<0.001
56	253 (181–354)	338 (215–533)	90 (67–122)[b]	<0.001
A/Johannesburg/82/96 (H1N1)				
0	18 (12–27)	60 (37–97)	29 (20–42)	0.001
28	437 (292–655)	795 (484–1,303)	192 (132–278)	<0.001
56	428 (291–630)	750 (461–1,219)	231 (163–320)[b]	<0.001
B/Harbin/7/94				
0	9 (7–12)	12 (8–19)	11 (8–15)	0.604
28	104 (65–166)	110 (61–199)	55 (37–83)	0.058
56	171 (119–246)[b]	122 (73–204)	76 (52–110)[b]	0.011

LTx, liver transplant recipients.
[a]95% confidence intervals are given in parentheses.
[b]Significant increase ($P < 0.05$) from day 28 to day 56.

shown in Fig. 1 and Table II, the antibody titers measured against this virus were significantly lower in all three study populations than those measured against the influenza A/H3N2 component of the vaccine, which was based on influenza virus A/Nanchang/933/95. The prevaccination GMT observed in the group of LTx recipients (GMT = 6) did not differ significantly from that in the control group (GMT = 6) and the group of cirrhosis patients (GMT = 8) ($P = 0.059$). Individuals in the control, cirrhosis, and LTx groups significantly responded to A/Sydney/5/97 after one vaccination, resulting in postvaccination titers of 27, 28, and 16, respectively. The response of the LTx group, however, was significantly lower compared with the responses measured in the control group or the group of patients with liver cirrhosis ($P < 0.001$). After the second vaccination, the GMT values in the three groups virtually did not change. The percentage of individuals in the LTx group (19.7%) with postvaccination HI titers ≥ 40 to influenza virus A/Sydney/5/97 was significantly lower after one vaccination compared with the control group (57.8%) and the cirrhosis group (51.4%). This percentage increased to 23.3% for the LTx group, but the difference compared with the two other groups remained statistically significant ($P < 0.002$).

In Vitro T-cell Proliferative Responses

After one influenza vaccination, the LTx patients showed a significant increase in their in vitro T-cell proliferative responses against influenza virus A/H3N2, A/H1N1, and B antigens (with *P* values of 0.034, 0.013, and 0.056, respectively) (Fig. 4). The average stimulation indexes further increased after the second vaccination, though the difference compared with the response after one vaccination was not statistically significant ($P = 0.500$ for A/H3N2, $P = 0.431$ for A/H1N1, and $P = 0.330$ for B antigen). Moreover, the percentage of individuals responding with stimulation indexes of 3.0 or higher increased after one influenza vaccination, from 8.0 to 45.8 for the influenza virus A/H3N2 antigen, from 20.8 to 41.7 for A/H1N1 antigen, and from 4.2 to 29.2 for influenza virus B antigen. After the second vaccination these percentages increased slightly.

In the control group, in vitro proliferative responses were observed after one influenza vaccination, which were significantly higher than before vaccination for the influenza virus A/H3N2 ($P = 0.040$) and B antigen ($P = 0.027$), but not for the A/H1N1 antigen ($P = 0.452$). In this group, a second vaccination did not lead to a significant increase of the in vitro T-cell response ($P > 0.400$). The response to the respective influenza virus antigens measured before and after one or two vaccinations tended to be higher in the control group than in the LTx patients. The differences found were not statistically significant ($P > 0.140$), however, with the exception of the response to A/H3N2 antigen before vaccination, which proved to be higher in the control group ($P = 0.050$).

DISCUSSION

In the present study, we have shown for the first time that adult LTx recipients respond to influenza vaccination with virus-specific antibody responses and in vitro proliferative T-cell responses, despite treatment with immunosuppressive drugs. Although vaccination leads to postvaccination HI titers ≥ 40 against all vaccine strains in the majority of these patients (>68%), the magnitude of the antibody response, expressed as GMT, was significantly lower than that observed in their healthy spouses and in the group of patients with liver cirrhosis. The latter group was included in the study as a control group of patients with liver disease who were not receiving immunosuppressive therapy. The percentages of LTx recipients with antibody titers ≥ 40 against all three vaccine components were lower after one vaccination (significantly for the influenza A strains but not for the B strain). After the second vaccination, an increase was observed in the percentage of individuals showing titers ≥ 40 against all the vaccine strains (> 80%), and the differences in percentage of individuals with titers of ≥ 40 to

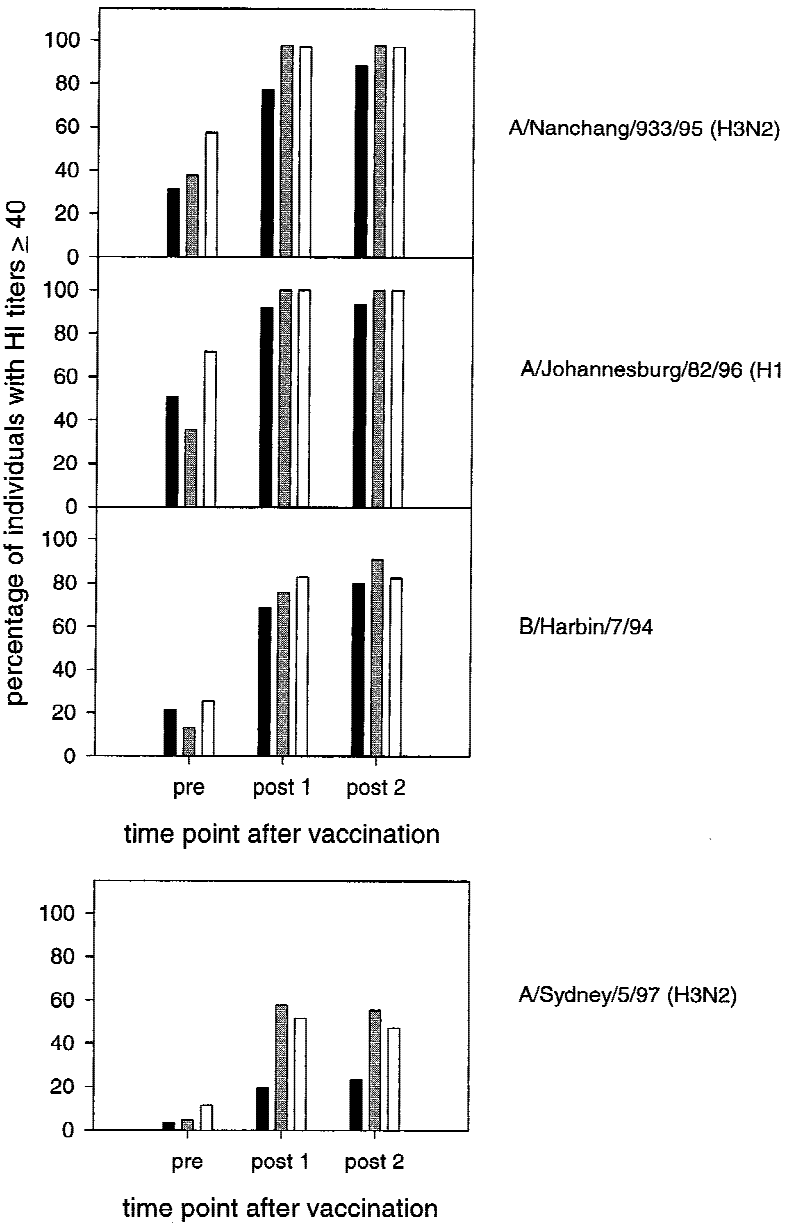

Fig. 2. Percentages of individuals with HI serum antibody titers ≥ 40, before and after one or two vaccinations against the three vaccine strains and the epidemic strain A/Sydney/5/97 in the group of LTX recipients (black bars), the control group (grey bars), and the group of cirrhosis patients (white bars).

A/Nanchang/933/95 and A/Johannesburg/82/96 were no longer statistically significant.

The percentages of LTx recipients responding with greater than or equal to fourfold titer rises upon vaccination were significantly lower than in the control group. This percentage tended to be lower in the group of cirrhosis patients as well, which most likely can be attributed to the higher level of preexisting antibodies before vaccination in this study group. In immunocompetent individuals, an HI titer ≥ 40 generally is considered to correlate with protection [Kilbourne et al., 1973; Briggs et al., 1980]. It is not clear at present whether this is also the case in immunocompromised individuals. The protection observed in the former group may be related directly to the presence of HI antibodies, which have been shown to neutralize the virus. It cannot be excluded, however, that vaccine-induced, cell-mediated memory response would contribute to protective immunity. Therefore, HI antibody levels might correlate with, rather than be solely responsible for, the protection induced by vaccination. If this was indeed the case, HI antibody levels might not correlate with protective immunity in LTx recipients, in whom cyclosporine treatment has an especially severe suppressive effect on T-cell responses. Within the group of LTx recipients, no significant differences were noted between patients treated with different regimens of immunosuppressive therapy (data not shown). The study design and the limited group sizes, however, may not have allowed the identification of such differences.

In spite of immunosuppressive treatment, an increase in virus-specific in vitro proliferative T-cell re-

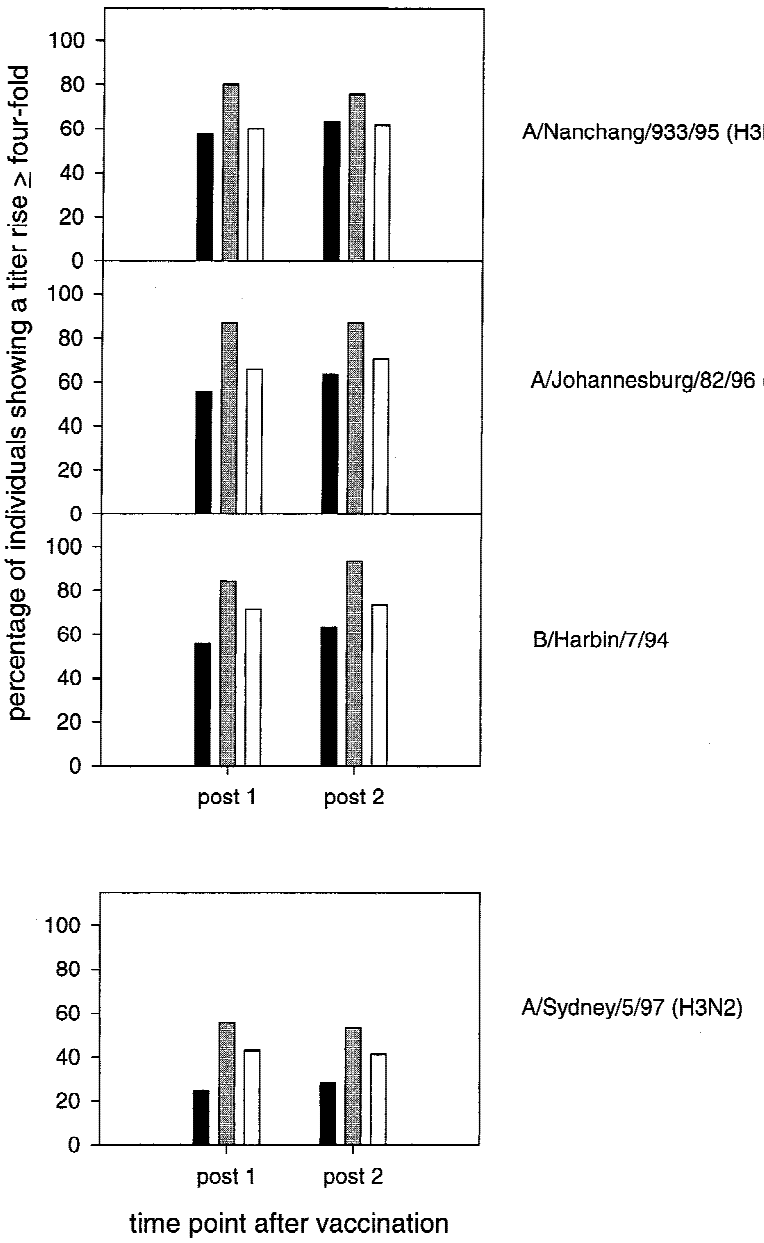

Fig. 3. Percentages of individuals responding after vaccination with titer rises of at least four-fold against the vaccine strains and the epidemic strain A/Sydney/5/97 in the group of LTx recipients (black bars), the control group (grey bars), and the group of cirrhosis patients (white bars).

TABLE II. Geometric Means of Hemagglutination-inhibiting Antibody Titers Against Epidemic Influenza Virus Strain

Days after vaccination	Controls[a]	Cirrhosis[a]	LTx[a]	*P* (ANOVA)
A/Sydney/5/97 (H3N2)				
0	6 (5–7)	8 (6–11)	6 (5–8)	0.059
28	27 (18–41)	28 (17–47)	10 (8–14)	<0.001
56	25 (17–37)	27 (16–45)	11 (8–16)	0.002

LTx, liver transplant recipients.
[a]95% confidence intervals are given in parentheses.

sponses was observed after vaccination, which coincided with the increase of virus-specific antibody responses. No direct correlation between the levels of increase in the respective assays was established for individual vaccinees (not shown). Furthermore, the proliferative T-cell responses in the group of LTx recipients tended to be lower than in the control group, though this difference was not statistically significant. In addition to the antibody responses directed against the vaccine strains, the response to the prototype

TABLE III. Proportions of Previously Unprotected Subjects with Postvaccination Titer ≥ 40

Influenza strain	Study group			
	Control	Cirrhosis	LTx	P (χ^2)
A/Nanchang/933/95	27/28 (96.4%)	14/15 (93.5%)	28/42 (66.7%)	0.00318
A/Johannesburg/82/96	28/29 (96.6%)	10/10 (100.0%)	21/30 (70.0%)	0.00560
B/Harbin/7/94	28/39 (71.8%)	20/26 (76.9%)	29/48 (60.4%)	0.28898
A/Sydney//5/97	24/43 (55.8%)	14/31 (45.2%)	10/59 (16.9%)	0.0014

LTx, liver transplant recipients.

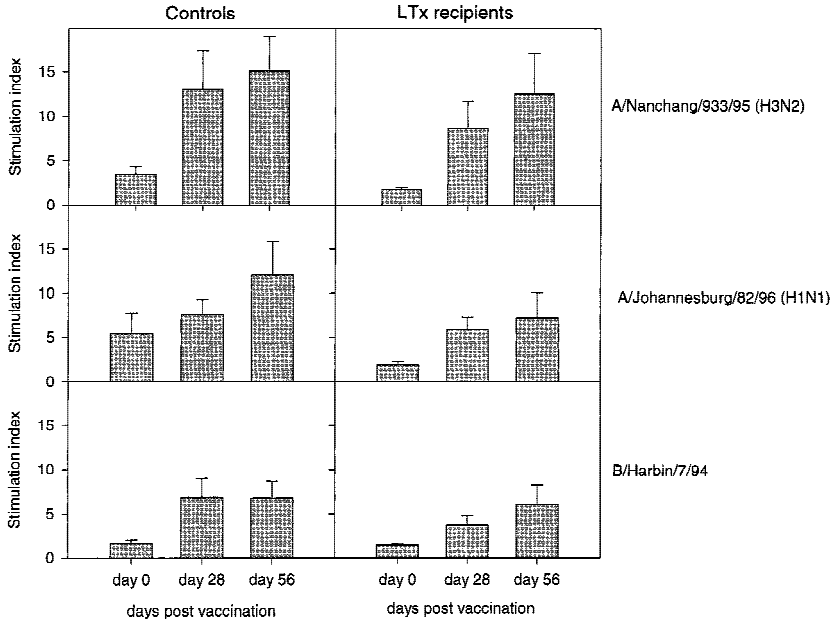

Fig. 4. In vitro proliferative responses of peripheral blood mononuclear cells (PBMCs) obtained from 25 LTx recipients and 23 healthy control individuals before and after one or two vaccinations. In all, 10^5 PBMCs were cultured in the presence of 300 ng membrane glycoprotein of the respective vaccine strains. The responses are represented as the average stimulation indexes ± SEM. The time points of the vaccinations are as indicated in Fig. 1.

strain, which contributed to the epidemic in the 1997–1998 influenza season, was tested. This influenza virus strain, A/Sydney/5/97 (H3N2), was shown to be antigenically different from the vaccine strain A/Nanchang/933/95 [Anonymous, 1997].

Since it has been reported that treatment with cyclosporine preferentially inhibits the primary immune response to new antigens [Charan et al., 1986], it could be anticipated that the response to the influenza virus A/Sydney/5/97, representing a virus strain with novel epitopes, would be relatively lower in LTx recipients compared with the response in control groups. A similar response was found in elderly people (>80 years of age), whose antibody response to influenza virus A/Sydney/5/97 upon vaccination with A/Nanchang/933/95 strain was relatively poor [de Jong et al., in press). Indeed, the group of LTx recipients in our study showed a lower heterologous antibody response to influenza virus A/Sydney/5/97 compared with control subjects and cirrhosis patients, confirming our expectation. The poor reactivity against influenza strain A/Sydney/5/97 after one vaccination (18.0% of individuals with titers ≥ 40) or two vaccinations (23.3% of individuals with titers ≥ 40) indicates that a mismatch of the vaccine strain with the epidemic strain may have significant consequences for vaccine efficacy, especially in immunocompromised patients.

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